EXPERIMENTS ON LIGIA IN BERMUDA

IV. THE EFFECTS OF HEAVY WATER AND TEMPERATURE [1]

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Three previous papers of this series (Barnes, 1932, 1934, 1935) have described salt effects and space orientation of the littoral isopod *Ligia baudiniana* in Bermuda. Since this species of *Ligia* is a sub-tropical form living under fairly uniform temperature conditions, it was decided to establish the upper temperature range in air and in sea water and to determine the effect of immersion in concentrated heavy water, which is equivalent to a drop in temperature from the standpoint of energy content (*cf.* Eyring and Sherman, 1933; Barnes and Gaw, 1935; Barnes and Warren, 1935).

METHODS

For the study of lethal temperatures a freshly collected adult was placed in a bottle of approximately 300 cc. capacity immersed in the water of a thermostat warmed by a knife heater controlled by a mercury relay. Fifty cc. of sea water or filter paper moistened with sea water were placed in the bottle. Air was pumped continuously through a spiral of glass tubing in the thermostat to bring it to the required temperature before it passed through the bottle. The time of death was taken as the point when the animal ceased all motion and would not respond when the bottle was violently jarred. All specimens were returned to sea water at normal temperature (27° C.), but none recovered after this end point was reached. Although the thermostat was accurate to 0.1° C., the temperature of the specimen bottle dropped 0.5 − 1° C. for a few minutes after the specimen was introduced.

For the determination of the temperature characteristics of the gill-beat, the animal was placed in 2 cc. of sea water in a test tube containing the bulb of a thermometer and a tube through which air was bubbled continuously. The test tube was immersed in a beaker of water at 27° C. and pieces of cracked ice were added with forceps to lower the temperature. Since only 2 cc. of 96 per cent heavy water were available, the experiment had to be carried out on a small scale

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[1] Contributions from the Bermuda Biological Station for Research.

(including the controls). The temperature was constant to within 0.4° C. during one reading (time for 10 beats of the pleopods).

Further Experiments with Salt Solutions

Sea water was used as the immersion medium in the temperature studies although animals will not live indefinitely in this medium. Artificial sea water in which the two most toxic ions K and Mg were reduced did not give better results than natural sea water (Table I). It will be seen from the table, however, that artificial sea water with half the normal Mg content is more favorable than artificial sea water with the normal amount of Mg. As was pointed out in a previous paper (Barnes, 1935), the K content of artificial sea water has to be increased four-fold to produce easily recognized toxic effects. Three seasons work with *Ligia* has shown the great variation that occurs in the survival times of specimens in sea water or salt mixtures similar to sea water and it is now evident that many tests have to be run with each solution, including a parallel series of sea water controls with animals collected at the same time and place, and the probable error and coefficient of variation worked out (Table I). The probable error is calculated from Peter's formula and the coefficient of variation is expressed as 100 × P. E. divided by the mean. The earlier results with toxic solutions of single salts are clear-cut and show little variation, but the great variation in ternary salt solutions can be seen in the following sample giving survival times of 14 animals each in 100 cc. of sea water: 5.5, 5.5, 5.5, 5.5, 14.5, 14.5, 14.5, 21, 29.5, 31, 31, 53.2, 192 and 240 hours respectively.

Table I

Longevity of Ligia in salt mixtures

Experiment No.	Solution *	Average longevity	Coefficients of variation	Maximum longevity	No. specimens tested
		hours		*hours*	
1	100 NaCl, 2.5 $CaCl_2$, 1 KCl, 11.6 $MgSO_4$	23± 2.3	10	96	36
	sea water control	61± 9.0	14	214.5	36
2	100 NaCl, 2.5 $CaCl_2$, 1 KCl, 5.8 $MgSO_4$	40± 4.0	10.3	288	63
	sea water control	52± 6.4	12.2	336	63
3	100 NaCl, 2.5 $CaCl_2$, 1 KCl	20± 2.5	12.4	89.5	32
	sea water control	82±12.4	15	288	32
4	100 sea water + 1 KCl	39± 7.8	20	288	17
	sea water control	57±11	19.2	216	16

* The numbers indicate cc. of 5/8 M solutions.

SURVIVAL IN AIR AND IN WATER AT HIGH TEMPERATURES

Ligia baudiniana dies in about one hour if placed in aerated sea water at 38° C. (Table II), but at this temperature in air (over filter paper moistened with sea water) it survives so long that the longevity could not be determined. At 42° C., however, specimens in air die in slightly less than an hour and 50° C. is rapidly fatal in both water and air. If a specimen is slowly heated in 50 cc. of sea water with a temperature rise of about $\frac{1}{2}$° C. per minute, the gill-beat becomes irregular at 39 − 40° C. and death occurs at 40–42° C. The Arrhenius equation, velocity $\alpha e^{-\mu/RT}$, was applied to the data for the rate of killing in air and yields a value of μ, the temperature characteristic, of 56,400 calories.

TABLE II

Survival times in sea water and in air at high temperatures

Sea water			Air		
Temperature	Average survival	No. specimens tested	Temperature	Average survival	No. specimens tested
° C.	*minutes*		° C.	*minutes*	
38	61.8 ± 4.7	15	42	52.7 ± 5.7	14
44	7.6 ± 1	8	44	20 ± 2	19
50	0	8	45	11.5 ± 3	6
			50	3 ± 0.3	16

TEMPERATURE CHARACTERISTICS FOR THE GILL-BEAT IN "HEAVY" SEA WATER

The flapping of the pleopods is regular over a temperature range of about 10–38° C., but the frequency is too great above 28° for accurate timing with a stop-watch. In a typical specimen the time for ten beats increases from 2.5 seconds at 26° to 7 seconds at 10°. The gill-beat begins to be irregular at 10° and stops below this critical temperature. After exposure to 10° or below the frequency remains lower than normal when the temperature is raised (Fig. 1). Therefore, the data used in calculating the temperature characteristic were obtained from readings taken as the animal was cooled from 27° to 10°. As will be seen from Fig. 1, the readings on the upward temperature curve are all lower than those in the downward direction. For the heavy water experiments 2 cc. of sea water were evaporated down and the salts redissolved in 2 cc. of 96 per cent D_2O (specific gravity 1.1037). The same procedure was used for the controls except that the salts from the evaporated sea water were taken up in ordinary distilled water. The frequency of the gill-beat immediately falls in "heavy" sea water. Thus at 20° C. a typical determination for the time for ten beats was 3.8 seconds in H_2O and 4.5 seconds in D_2O. In Fig. 1 it will be seen that this lower frequency obtains throughout the entire temperature range in heavy water and that exposure to 10° is followed by a further reduction in rate as in ordinary sea water.

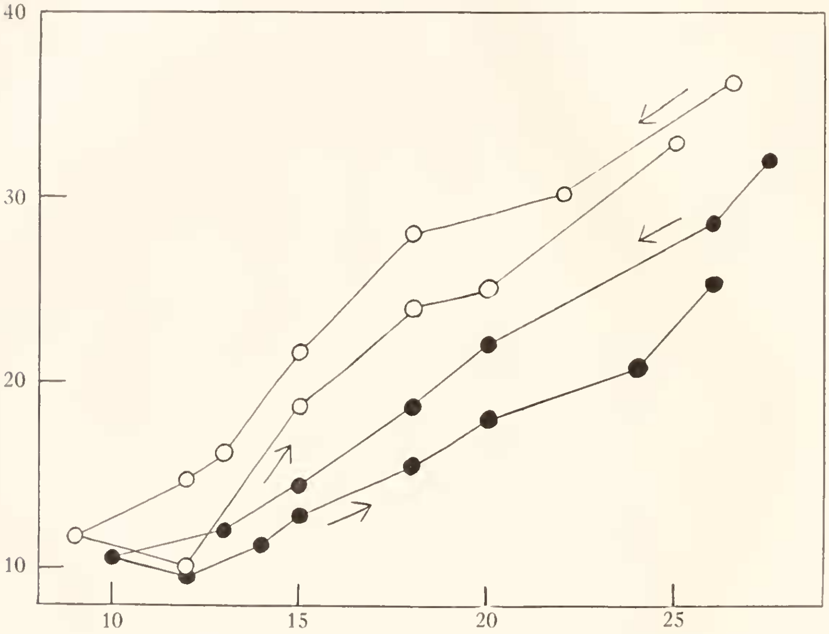

FIG. 1. Rate of beating of the pleopods of *Ligia* as a function of temperature. Ordinates, rate expressed as 100 divided by the time for 10 beats. Abscissæ, temperature in degrees centigrade. Open circles represent a specimen in ordinary sea water. Black dots represent a specimen in sea water containing 96 per cent heavy water. The rate was taken at the higher temperature first and then at progressively lower temperatures to about 10° where the beat becomes irregular. Then the temperature was raised, as indicated by the arrows. Each point is the average of five determinations of the time for 10 beats. Each run from 28° to 10° or 10° to 28° required about 40 minutes.

The data for both ordinary and "heavy" sea water are plotted in Fig. 2 according to the Arrhenius equation

$$ln\frac{R^2}{R^1} = \frac{\mu}{2}\left(\frac{1}{T^1} - \frac{1}{T^2}\right),$$

where R^1 is the rate at T^1 and R^2 is the rate at T^2. It appears that the slope of the line changes sharply at 16.5° C. in H_2O sea water and at 19.5° C. in D_2O sea water. The frequency is greatly reduced in heavy water but it is interesting to observe that the lines for both media are parallel. In ordinary sea water the temperature characteristics are: 16,000 calories for the interval 10–16.5° C. and 8,400 calories between 16.5 and 27° C. For "heavy" sea water the μ values are; 15,000 calories, 10–19.5° C. and 8,400 calories between 19.5° and 27° C. The significance of these findings will be discussed in the sequel. There were no signs of injury during the hour and a half run in heavy water and the reduction in frequency of the gill-beat was reversible after return to ordinary sea water. Thus a specimen in which the time for ten beats was 5.5 seconds at 17° in D_2O showed a typical rate of ten beats in 4.2 seconds at 17° after return to sea water.

It will be noted in Fig. 2 that the scatter of the plotted points is much greater in H_2O sea water than in D_2O sea water. Owing to the scarcity of concentrated heavy water, the determinations were made from two specimens. Although many animals were used in ordinary sea water, the data from only two typical specimens are plotted in Fig. 2 as a basis for comparison with the D_2O results. This procedure is

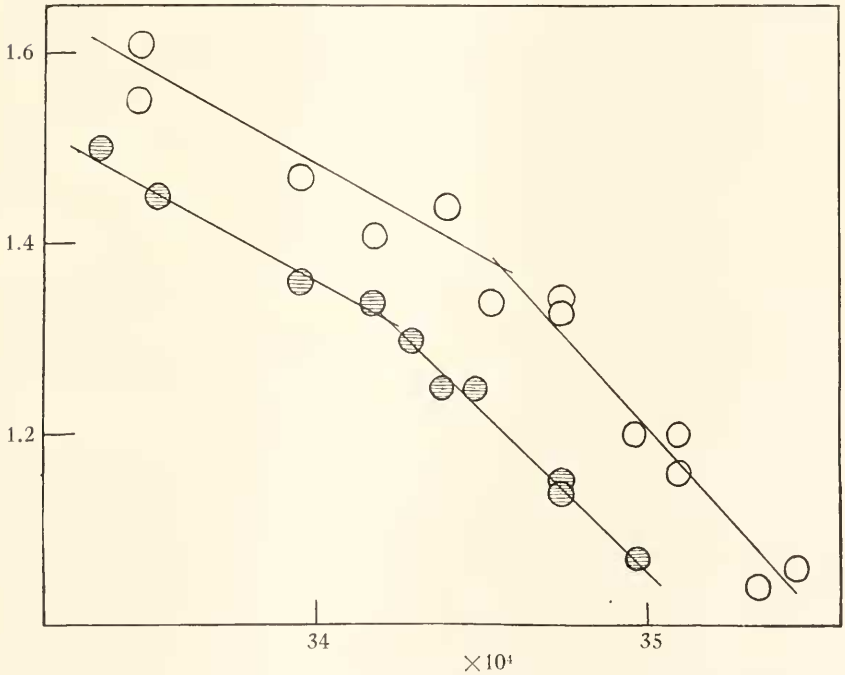

FIG. 2. Relation between the log of the rate of gill-beat in *Ligia* (ordinates) and the reciprocal of the absolute temperature (abscissæ). Open circles represent data from two specimens in ordinary sea water. Shaded circles represent readings from two individuals in sea water containing 96 per cent heavy water. In the upper graph the lines intersect at 16.5° C., in the lower at 19.5° C. (See text.)

justified by the fact that in ordinary sea water the temperature characteristics are the same as those obtained by Numanoi (1935), who used hundreds of specimens of the closely allied *Ligia exotica*. The specimens used in the 96 per cent D_2O measured 13 and 14 mm. in length (not counting uropods and spines) which were large in comparison to the 2 cc. of water, so that the concentration in the tissues was probably about 80 per cent heavy water.

DISCUSSION

Evidence is accumulating that the inability of *Ligia* to survive when immersed in sea water is not entirely determined by the relative proportions of the chief cations. However, it is interesting to note that a reduction by half of the Mg content of artificial sea water permits greater longevity in that medium but a synthetic sea water of greater sustaining power than natural sea water has not yet been found.

The studies of the rate of killing of the isopod at high temperatures indicate an abrupt transition from the rate of killing curve to the ordinary curve of life duration as found by Brown and Crozier (1927) in cladocerans. The lethal temperatures for *Ligia* in Bermuda do not appear to be higher than those for organisms in the temperate zone, but the minimum temperature for the gill-beat, 10° C., is four degrees higher than that of *Ligia exotica* in Japan (Numanoi, 1933). The high temperature characteristic for the rate of killing of *Ligia* at high temperatures is similar to values obtained from such processes as the coagulation of proteins by heat (Buchanan and Fulmer, 1930, p. 52). The more rapid lethal effect of high temperature in water than in air is a common phenomenon and probably not connected with the greater longevity of *Ligia* in air at ordinary temperatures (25–28°).

The temperature characteristic of 16,000 calories obtained for the gill-beat is one repeatedly encountered for respiratory processes (Numanoi, 1933) and is associated with the catalysis of oxidation by iron (Crozier, 1924). The μ values obtained by Numanoi (1933) for the gill-beat of *Ligia exotica* in Japan, i.e., 15,200–17,000 calories between 6–16° C. and 7,000–8,200 calories between 16 and 30° C., are precisely the same for *Ligia baudiniana* in Bermuda. These two values occur together frequently for respiratory processes.

The application of the Arrhenius equation to the data for the gill-beat in heavy water should throw light both on the mechanism of action of the heavy hydrogen isotope and on the validity of the Arrhenius equation in biological systems. Crozier (1935) has recently discussed modifications of temperature characteristics from which it is clear that if the several discrete magnitudes of μ persist in spite of modifications of the temperature graph, it may be concluded that the temperature characteristic represents the molecular energy of activity of a specific chemical process. Now it will be seen in Fig. 1 that in spite of the great reduction in frequency by heavy water, the rectilinear relation is not destroyed and the same discrete values of μ can be recognized as for the process in ordinary water. Not only so, but the data from the heavy water series fit the equation even more closely in that the points all lie on the line.

It appears that the heavy water reduces the frequency of the gill-beat without changing the fundamental regulating mechanism. A similar effect is seen in the action of adrenalin on the heart (Crozier, 1926), in which the frequency is greatly increased but the temperature characteristic is not changed. Urey and Teal (1935), when discussing the energies of activation of chemical reactions in heavy water, point out that no difference in the activation energies need be expected in reactions in which hydrogen is not directly involved. On the other hand, the reaction between deuterium and bromine to form DBr has an activation energy of 19,870 calories which is 2,100 calories higher than that for the reaction $H + Br = HBr$, the difference being about equal to the difference in the zero point energy of the two hydrogen isotopes.

It remains to be seen if there are biological processes in which the temperature characteristic is changed greatly in heavy water. The rate of pulsation of the contractile vacuole in *Paramecium* (Barnes and Gaw, 1935) has three values of μ over the temperature range in ordinary water i.e., below 16°, 24,000; 16–22°, 17,000; and above 22, 14,000; but in heavy water the value is 22,000 over the entire range. The rate of pulsation of the turtle's auricles (unpublished data), like the gill-beat in *Ligia*, yields a μ value of 16,000 in both D_2O and H_2O. It is hoped that further temperature studies in heavy water will afford new and significant information concerning the regulation of physiological processes.

The effect of heavy water on the rate of the gill-beat is equivalent to a drop in temperature of 3–4° C. This is also seen in the critical temperature or point in the graph (Fig. 2) where the slope of the line changes. The line breaks at a point 3° higher in the plot for the heavy water experiment. It is proposed that the effect of heavy water on a physiological process should be expressed as an "*equivalent drop in temperature*" of a specific number of degrees. This is similar to a new method of expressing the configuration of the water molecules in a dilute solution of strong electrolytes proposed by Bernal and Fowler (1933)—the "structural temperature" or that temperature at which pure water would have effectively the same inner structure as the given solution.

SUMMARY

1. The survival of *Ligia* in artificial sea water is doubled if the Mg content is reduced by one half.

2. In spite of the fact that *Ligia* cannot survive long periods in natural sea water, no artificial sea water has been found to be a more favorable medium.

3. Exposure of about one hour to sea water at 38° C. or to moist air at 42° C. is fatal to *Ligia*.

4. In natural sea water the beating of the pleopods of *Ligia baudiniana* yields temperature characteristics of 16,000 calories between 10 and 16° C. and 8,400 calories between 16 and 28° C. These are typical of respiratory processes.

5. The reduction in the frequency of the gill-beat in sea water containing 96 per cent heavy water is equivalent to that produced by a drop in temperature of about 3–4° C. Similarly the critical temperature in heavy water is shifted three degrees higher.

6. In sea water containing 96 per cent heavy water the gill-beat yields temperature characteristics of 15,000 calories between 10 and 19 and 8,400 calories between 19 and 28° C.

7. It is concluded that the lower energy content of heavy water slows down the beating of the gills but does not change the fundamental regulatory mechanism.

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